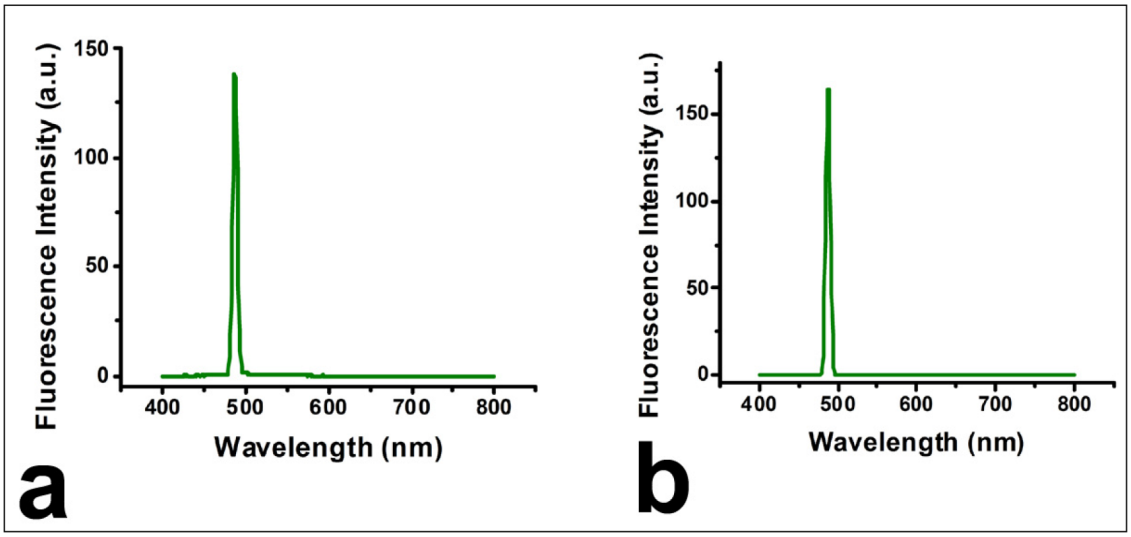
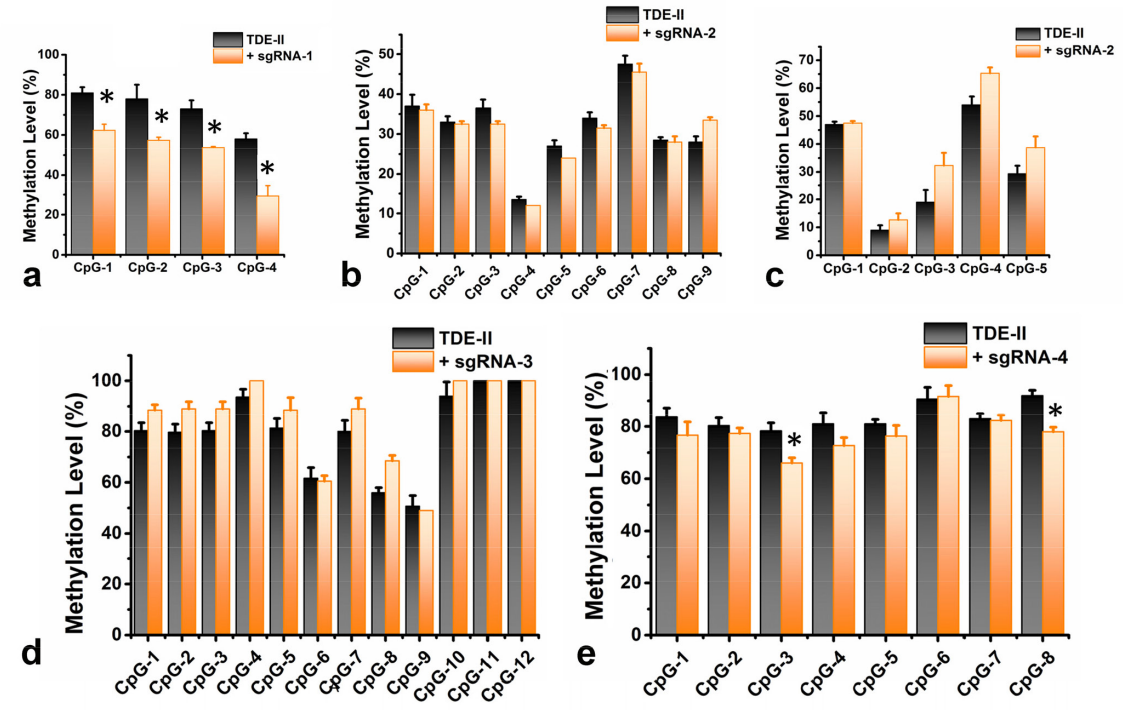
CRISPR-dCas9 mediated TET1 targeting for selective DNA demethylation at *BRCA1* promoter

SUPPLEMENTARY DATA

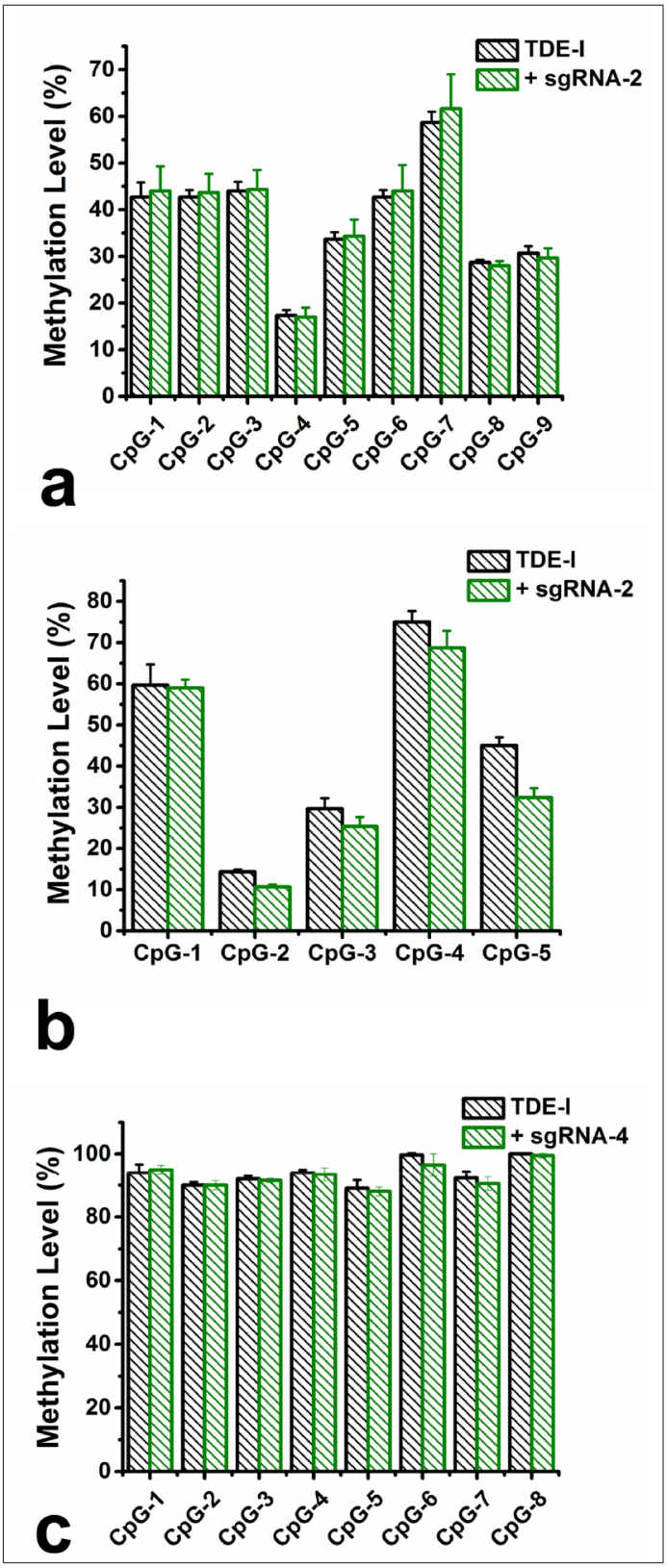
See Supplementary Sequence File: 1



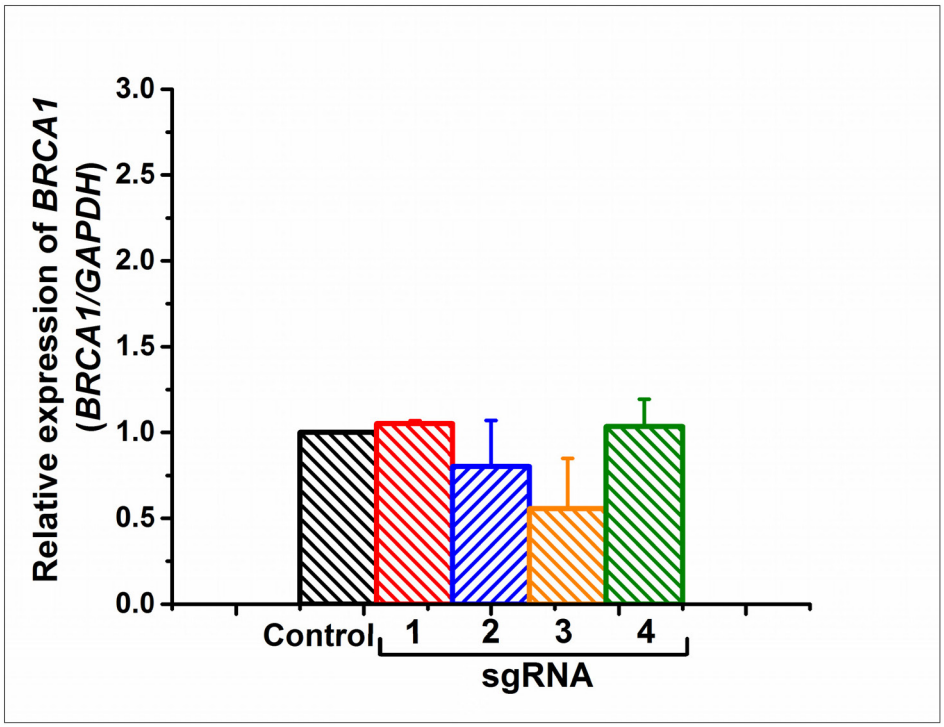
Supplementary Figure S1: The total protein was extracted from the co-transfected (combination of TET1-dCas9 plasmids and sgRNAs) cells, and the emission spectra of the fluorescent tags (EGFP) in the fusion proteins were recorded using a fluorimeter. A slight shift in EGFP emission also suggests the possible formation of TET1-dCas9-EGFP fusion proteins namely TDE-I and TDE-II. Representative emission spectra is presented for TDE-I in HeLa **a.** and MCF7 **b.** cell free extracts.



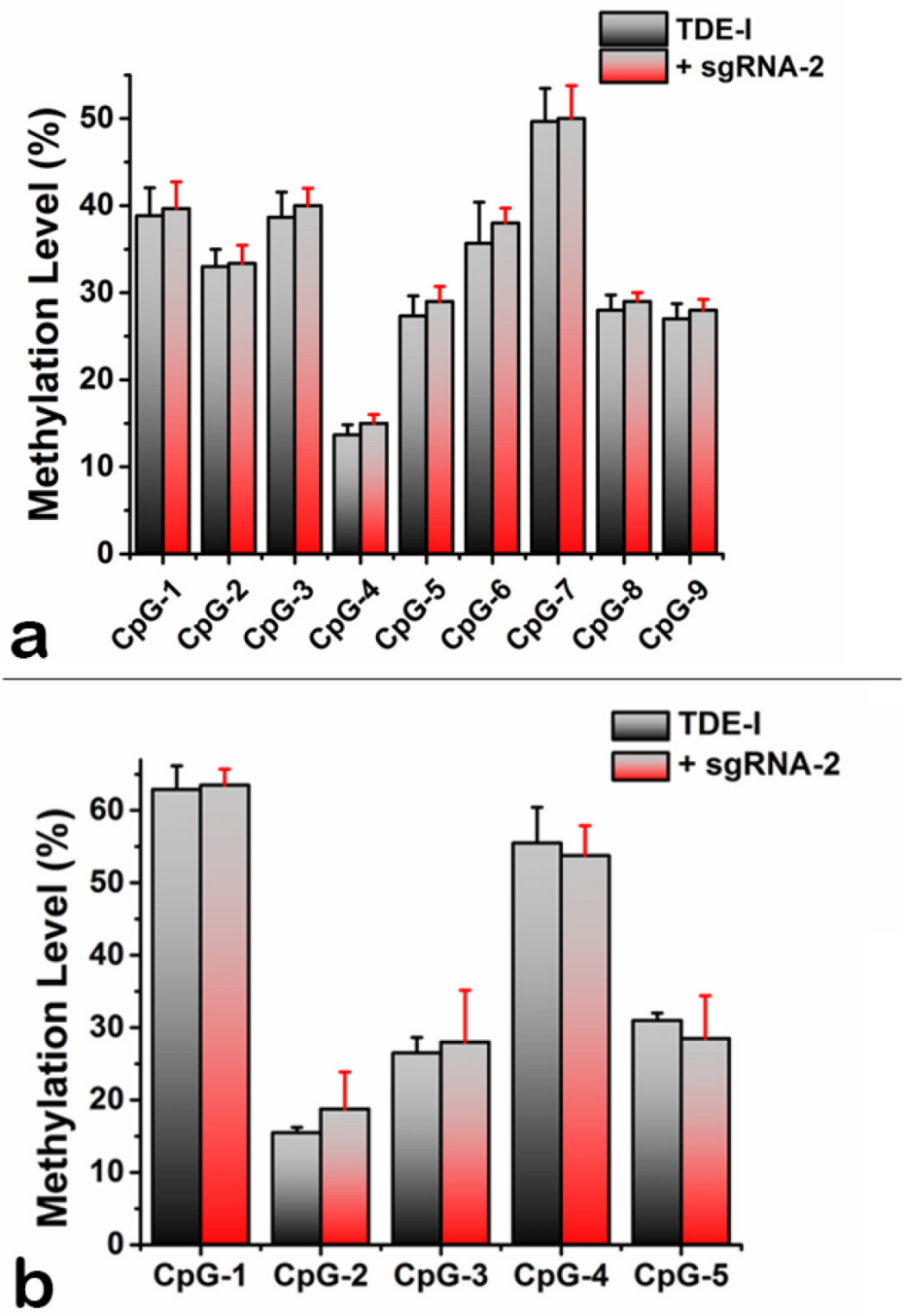
Supplementary Figure S2: Quantitative determination of DNA demethylation levels at the target CpG sites in *BRCA1* promoter. The levels of DNA methylation were determined by pyrosequencing in HeLa cells, treated with TDE-II and different combinations of sgRNAs (in orange), compared to only TDE-II treated ones (in black) (**a-e**). A p value of < 0.05 was considered statistically significant for all the obtained data.



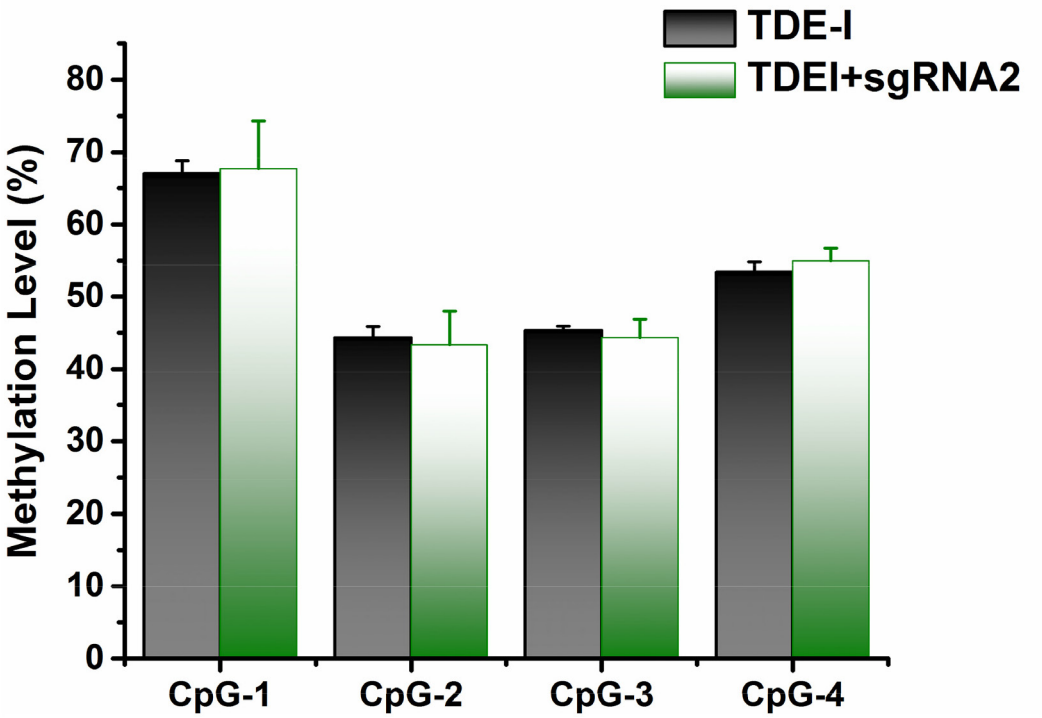
Supplementary Figure S3: Quantitative determination of DNA demethylation level at the target CpG sites in *BRCA1* promoter. The levels of DNA methylation were determined by pyrosequencing in MCF7 cells, treated with the combination of TDE-I and sgRNA-2 (**a, b**), and the combination of TDE-I with sgRNA-4 in MCF-7 cells.



Supplementary Figure S4: The gene expression of *BRCA1* was determined with qPCR analysis followed by the co-treatment with TDE-II and different combinations of sg-RNAs in HeLa cells.



Supplementary Figure S5: Quantitative determination of DNA demethylation level at the target CpG sites in *BRCA1* promoter. The levels of DNA methylation were determined by pyrosequencing in HeLa cells, treated with the combination of inactive TET1 fusion protein and sgRNA-2 (**a, b**).



Supplementary Figure S6: Quantitative determination of DNA demethylation levels at the target CpG sites at global LINE-1 repeat sequence element. The levels of DNA methylation were determined by pyrosequencing in HeLa cells, treated with TDE-I and compared to the combination of TDEI + sgRNA-2 (**a-e**). A *p* value of < 0.05 was considered statistically significant for all of the obtained data

Supplementary Table S1: Primers used in this study for PCR amplification and sequencing analysis of the fusion-protein construct (TET1CD- dCas9-EGFP), all primers listed are 5' to 3'

TET1CD- dCas9-EGFP (TDE) fusion protein construct			
PCR primers			
Insert name		**Forward**	**Reverse**
TDE-I	**TET1CD**	atcgttGGTACCGCCACCATGGAACT GCCCACCTGCAGCTGTCTTGAT	atcgttGGATCCGAC CCAATGGTTAT AGGGCCCCGCAACGTG
	dCas9	ATCGTTGGATCC AGCGGAAGTACACCCGCA ATGGCTAGCCCCAAA AAGAAGAGGAAAGTG	atcctaTCTAGA ACCTACCTTGC GCTTTTTCTTGGGAG
TDE-II	**TET1CD**	atcgttGGTACC GCCACC ATGGAACTGCC CACCTGCAGCTGTCTTGAT	atcgttGGATCC GACCCAATGGTT ATAGGGCCCCGCAACGTG
	dCas9	atcgttGGATCC GCCACC ATGGCTAGCCCC AAAAAGAAGAGGAAAGTG	atcctaTCTAGA ACCTACCTTG CGCTTTTTCTTGGGAG
Sequencing primers			
Primer name	**Sequence (5' to 3')**	**Primer name**	**Sequence (5' to 3')**
TDE_SP1	GAGGGGTTTTATGCGATG	**TDE_SP6**	CGTGAACACTGAA ATCACCAAG
TDE_SP2	TCCAAGCTCTCCCTTACATGA	**TDE _SP7**	GTACAACGAGCT GACCAAGGTG
TDE_SP3	ACCTTAGGGAGT AACACTGAGACC	**TDE _SP8**	GTATTCTCCAGACCG TGAAAGTC
TDE_SP4	ACATTGATGAGTAT TGGTCAGACAG	**TDE _SP9**	CGCGAGGTG AAGGTGATTA
TDE_SP5	ATCCCATCTTCGGTAATATCGT	**TDE _SP10**	GAACTGCTGGGT ATCACCATTAT
Sequence (3' to 5')			
TDE_SP11	TGTACAGCTCGTCCATGC		

The restriction sites are represented in green, linkers in red, and the sites complementary to the inserts in black font.

Supplementary Table S2: Primers used in this study for PCR amplification and sequencing analysis of the fusion-protein dCas9- TET1CD (inactive)-EGFP, all primers listed are 5' to 3'

dCas9- TET1CD (inactive)- EGFP fusion protein construct			
	PCR primers		
	Insert name	**Forward**	**Reverse**
dCas9-TET1CD (inactive)-EGFP	**dCas9**	atcgttCCATGGCTAGCCCC AAAAAGAAGAGGAAAGTG	gctagcGGCGCGCCCACCTA CCTTGCGCTTTTTCTTGGGAG
	TET1CD (inactive)	atcgttGCTAGC AGCGGA AGTACACCCGCA ATGGAACTGCCCA CCTGCAGCTGTCTTGAT	atcctaCCTGCAGGGACCCAAT GGTTATAGGGCCCCGCAACGTG
	EGFP	atcctaCCTGCAGG AAGCGGAAGTACACCCGCA ATGGTGAGCAAGGGCGAGG	tctacaaaGCGGCCGCCTACT TGTACAGCTCGTCCATG

The restriction sites are represented in green, linkers in red, and the sites complementary to the inserts in black font.

Supplementary Table S3: Primers used in this study for the PCR amplification of *BRCA1* after bisulfite conversion of the HeLa genomic DNA. The target region was amplified in two fragments with 2 sets of primers.

BSP-PCR primers				
	Forward	**Reverse**	**Product size (bp)**	**Annealing Temp (Tm)**
Fragment-1 (F1)	**/5BiosG/**GGGTTAGT TAGGGGTGGGGTTA	CCTCTCCCTCCACACTTC	380	58 °C
Fragment-2 (F2)	TATTTTGGTAGTG TTGGAGGAGTT	**/5BiosG/**AACCACCCT AAAACTCACAAAAATTAAA	400	56 °C
	Pyrosequencing primers	**Target sequencing CpG sites (highlighted in yellow)**		
F1-S1	*CTCTCATCCTATCACTAAAA	CGATTTTTTTCGTG TTTTCGGATAGTTAATCG		
F1-S2	*CAATAACCAACT AAAAAACTCCTC	CGAGGTGATAACGTGTTAGT AGTTTTCGTTCGTTTTCGGCGTTT TTTCGGTTTTGGCGTTTATTTTGGTCG		
F2-S1	GTGTTGGAGGAGTTT	CGTTATTGCGTTGTGGGGGTTTTT TTTTGGGTTGGTCGAAGTTAGAG TCGGTTTTTTTTGTTTGCG		
F2-S2	TGTGGGAATTGGGGT	CGCGTAGCGTTCGTTAGTTAGCGCGAGTT TTAGGTGGGCGCGGGTTTAGCGGGT TTCGTATTTTCGGTTTCG		
F2-S3	GTTTAGGGTAGTTAGGGG	CGGGTTAGTAGTTGTAGAGGG TGCGTCGGGTTTTTTAGTATTG TCGGTTCGTTTGTATTTCGTTTGA ATTTTTATCGGGTTTTAGTCG		

Pyrosequencing was then carried out to quantify the methylation percentage at each CpG sites of the analyzed regions. All the primers listed are 5' to 3'. Bisulfite-PCR (BSP) primers were 5'-biotinylated in either forward or reverse primer.

*Sequencing primer binds to the reverse strand. The sequence of corresponding CpG sites of these sequencing primers hence can be read in the reverse direction of the actual sequence.

Supplementary Table S4: Primers used to study transcript quantification of *BRCA1* relative to the endogenous control GAPDH by qPCR

	Forward	Reverse
BRCA1	CAAGGAACCAGGGATGAAATCAG	ATGGCTCCACATGCAAGTTTG
GAPDH	CAGCCTCAAGATCATCAGCA	TGTGGTCATGAGTCCTTCCA

All the primers listed are 5' to 3'.

Supplementary Table S5: Primers used for qPCR to determine locus specific 5-hydroxymethylation (5-hmC) at the target site of *BRCA1* promoter

Treated with TDE-I plus sgRNA-2		
	Primer sequence	**Covered 5-hmC sites† (Amplicon size: 270 bp)**
Forward	GAGCCCTTCGTGTTCTGAGG	**GCGC**CGGAGAGTTGGAGAGTCTGTGGT TCAGAATGCGAGGTGACAACGTGCTAGC AGCCCTCGCTCGCTCTCG**GCGC**CTCCTCGGCCTT GGCGTCCATTCTGGCCGTGCTGGAGGA GCCCTTCAGCCCGCCACT**GCGC**
Reverse	GGTGTGGGAACTGGGGCT	

All the primers listed are 5' to 3'.